Responses to Public Comment on the Draft Reference Exposure Levels for Methylene Diphenyl Diisocyanate

Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

November, 2014

On July 4, 2014, the Office of Environmental Health Hazard Assessment (OEHHA) released the draft document, *Methylene Diphenyl Diisocyanate Reference Exposure Levels: Technical Support Document for the Derivation of Noncancer Reference Exposure Levels* to solicit public comment. Responses to comments received on the draft methylene diphenyl diisocyanate reference exposure levels (RELs) are provided here.

Background

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360(b)(2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8-hour and chronic RELs and was adopted in December 2008. The TSD presents methodology for deriving RELs. In particular, the methodology explicitly considers possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, Chapter 731, Statutes of 1999, Health and Safety Code sections 39669.5 *et seq.*). These guidelines have been used to revise the existing chronic REL of 0.7µg/m^3 for methylene diphenyl diisocyanate, and derive new acute and 8-hour RELs.

Commenters on the Draft RELs for methylene diphenyl diisocyanate (MDI)

Comments were received from the American Chemistry Council Diisocyanates Panel (ACC).

Responses to MDI Comments Received from ACC

ACC Comment 1:

Overview: Although the 2014 MDI REL document (54 pgs) prepared by OEHHA has expanded on the 2010 version (18 pages), the document's conservative bias remains largely intact and has not addressed the Panel's comments made in 2010. The key studies OEHHA identified for the RELs are reasonable. However, OEHHA has not taken into account the human studies in the scientific rationale justifying the selection of uncertainly factors (UFs). Specific issues are outlined below and supplemented with earlier ACC comments, as appropriate.

Response to ACC Comment 1:

The UFs used by OEHHA in deriving RELs for MDI are consistent with guidance presented in the Noncancer Technical Support Document (OEHHA, 2008). Further details on individual UFs used are discussed in the responses to comments below.

ACC Comment 2:

OEHHA states (Section 4, pg 5) ***"The urinary excretion peak of the MDI metabolite 4,4'-diphenylmethane diamine occurred 12-14 hrs after end of exposure."***
The document incorrectly states that 4,4'-diphenylmethane diamine is a metabolite of MDI. The document does however correctly state in the previous sentence that diphenylmethane diamines are the hydrolysis product of the MDI metabolites.

Response to ACC Comment 2:

The paragraph in question was modified to clarify that "MDI metabolites" are hydrolyzed in the urinary samples to form 4,4'-diphenylmethane diamine (MDA) for analysis, and that MDA is not specifically found in urine before hydrolyzation.

ACC Comment 3:

The role of metabolic enzymes (e.g., N-acetyltransferases (NATs) and glutathione transferases (GSTs) in neuroimmune sensitization (Section 4, pg 5)) is not apparent, calling into question the need to consider genotypic variations in enzyme systems.
The potential association between a genetic polymorphism in enzyme systems affecting MDI metabolism and a susceptibility to respiratory disease is uncertain (Redlich and Karol, 2002; Berode *et al.*, 2005; Littorin *et al.*, 2008). Many contradicting reports exist in

the literature and no clear conclusion emerges (Littorin *et al.*, 2008), even with data from the same study. MDI primarily exhibits portal of entry toxicity. The sequence of events leading to respiratory tract sensitization and, in some cases, diisocyanate asthma is likely related to the dose to the epithelial tissues of the respiratory tract. Health outcomes reliant on direct interaction of MDI with receptors in epithelial tissues would not be affected by a genetic polymorphism in metabolic enzymes. The initial reaction of MDI with a nucleophile such as glutathione does not require catalysis by an enzyme system. MDI depositing at the liquid-air interface of the respiratory tract encounters the extracellular glutathione-rich liquid lining of the respiratory epithelium and is expected to form covalent bonds with glutathione, likely resulting in bis-glutathione adducts similar to those reported for toluene diisocyanate (Day *et al.,* 1997).

The major metabolic pathway for MDI involves N-acetylation and various stages of oxidation of the methylene bridge (Gledhill *et al.,* 2005). The most likely precursor of these metabolites is the bis-glutathione adduct of MDI. Although methylene dianiline (MDA) could be a candidate precursor, MDA was not found *in vivo* following inhalation exposure to MDI (Gledhill *et al.,* 2005) nor *in vitro* in the reaction of MDI to N-acetyl-L-cysteine (Moorman *et al.,* 2006). Interestingly Reisser *et al.* (2002) found the mono-glutathione adduct of MDI to be significantly more stable than the bis-adduct, thus allowing for N-acetylation to occur without complete hydrolysis to MDA. Because the formation of this conjugate is not enzyme mediated, genetic polymorphism is not expected to affect adduct formation**.** Thus, genotypic variation in MDI metabolic enzymes is not a relevant consideration for development of RELs for MDI. The conclusion of Littorin *et al.* (2008), "[t]he information on associations between genes and isocyanate-induced risk is limited and not consistent" should be included in the "Methylene Diphenyl Diisocyanate Reference Exposure Levels Technical Support Document, Section 4.

Response to ACC Comment 3:

We disagree with the major point of the comment that genetic polymorphisms in metabolic enzymes including GSTs and NATs are not relevant in the disease process resulting from MDI exposure.

Granted, the pathogenesis of diisocyanate-induced asthma is a complex process and still largely unknown. However, diisocyanates or their metabolites may react with intracellular glutathione (GSH), either directly or after catalysis by the GSTs. Thus, GSTs may help facilitate the reaction of GSH with MDI. Piirila et al. (2001) notes that enzymes of the glutathione S-transferase (GST) supergene family can utilize a wide variety of products of oxidative stress as substrates and are thus critical in the protection of cells from reactive oxygen species (ROS). Exposure to diisocyanates causes respiratory symptoms characterized by airway inflammation, eosinophilia, and local formation of ROS. Accordingly, the observed wide genetically-based individual variations in the GST enzyme activities are likely modifiers of susceptibility to diisocyanate-induced asthma. Individual capability to tolerate oxidative stress varies, possibly due to genetic factors. Inability to detoxify ROS could therefore lead to

inflammatory process, activate bronchoconstrictor mechanisms and cause asthmatic symptoms.

Also noted by Piirila et al. (2001), diisocyanates may react with proteins, possibly via GSH conjugates, to form protein conjugates. The protein conjugates may be immunogenic, and the formation of hapten complexes may give rise to immunological reactions. Therefore, in the presence of decreased GSH conjugation related to deficient GST genes, impaired immune response could also be suspected.

Finally, several researchers have observed that genetic variants of antioxidant defense genes for GSTs and NATs are associated with increased susceptibility to diisocyanate-induced asthma (Yucesoy et al., 2012; Piirila et al., 2001; Wikman et al., 2002). This information is presented in Section 6.4 (Toxicogenomics) of the MDI REL document. However, the statement in the comment that *"[t]he information on associations between genes and isocyanate-induced risk is limited and not consistent"* is partially true. We will add sentences to Section 6.4 to note this fact: *"The information on associations between genes and isocyanate-induced risk is currently limited and sometimes inconsistent results were obtained between studies. Table 9 presents the positive associations researchers have found between gene variants and increased susceptibility to diisocyanate-induced asthma."*

ACC Comment 4:

The rationale for linking MDI metabolism to potential health effects (Section 4, pg 5) is not clear.
OEHHA mentions that the predominant toxicological response produced by inhalation exposures to MDI is immune responses, an effect that can be explained by the direct interaction of MDI with respiratory tract tissue (*e.g.,* TRPA receptors, nucleophiles) to initiate sensitization. Metabolism of MDI, a highly reactive chemical, is not required for its participation in the sensitization process nor is there any evidence that pulmonary metabolism contributes significantly to the small fraction (~ 10%) of the inhaled dose that appears systemically as an acetylated and/or oxidized metabolite (Gledhill *et al*., 2005). The Diisocyanates Panel (Panel) notes that (a) available data collected by multiple investigators fail to link metabolic enzymes to MDI-induced immune responses (*See* 1.b. above), and (b) metabolism was not considered to play a significant role in the rat nasal lesions caused by acrolein, another reactive direct-acting chemical, when OEHHA derived the 8-hr and chronic RELs for same.

As it has for other reactive chemicals (e.g., acetaldehyde, acrolein, formaldehyde) OEHHA should indicate that MDI causes portal of entry effects and that available data have been unable to show that metabolism contributes in any significant way to the immune responses effects caused by MDI.

Response to ACC Comment 4:

We could not find the statement made by OEHHA in Section 4, page 5, that "the predominant toxicological response produced by inhalation exposures to MDI is immune responses…" However, we say at the beginning of Section 5: "As is the case with other diisocyanates such as toluene diisocyanate (TDI), MDI has the capacity to cause sensitization of the neuroimmune system." What we do say in Section 1 is that the critical effect is in the respiratory system. We say this because the data suggests there is more than one pathway of injury caused by MDI in the respiratory system (i.e., an inflammatory response likely from direct action on the lung tissue, as discussed in the acute REL derivation and an immune system response from repeated exposure resulting in sensitization).

As noted in the previous comment, researchers suggest diisocyanates or their metabolites may react with intracellular glutathione (GSH), either directly or after catalysis by the GSTs. Thus, GSTs may help facilitate the reaction of GSH with MDI. GSTs also appear to have an important role in detoxifying the ROS generated from reaction of diisocyanates with tissue and proteins.

We also point out in our response to Comment 3 that Piirila et al. (2001) suggests diisocyanates may react with proteins, possibly via GSH conjugates, to form protein conjugates. The protein conjugates may be immunogenic, and the formation of hapten complexes may give rise to immunological reactions. Therefore, in the presence of decreased GSH conjugation related to deficient GST genes, impaired immune response could also be suspected.

Recent work by Wisnewski et al. (2013) indicates that GSH can act as a "shuttle" for MDI. Once MDI-GSH is absorbed, MDI-albumin conjugates are generated via GSH-mediated transcarbamoylation, which exhibit distinct changes in conformation and charge. These MDI-albumin conjugates were specifically recognized by serum IgG of MDI workers with diisocyanate-induced asthma, suggesting one possible pathway for MDI in promoting immune responses.

Thus, it would be premature for OEHHA to say that the metabolic pathway for MDI is not linked to immune system responses.

ACC Comment 5:

OEHHA (Section 5.2, pg 9) assumes that purported asthma-like symptoms observed in school children were due to a MDI exposure (Jan *et al.,* 2008). However, the reported symptoms are more likely due to xylene, a known CNS depressant and upper respiratory tract irritant that was used as a solvent for the applied MDI.

The Panel notes that (a) air monitoring was not conducted for either volatile organic compounds or MDI, and (b) despite the claim by Jan *et al.*, an earlier work mentioned by the authors did not detect MDI near polyurethane tracks up to a week after application. Examination of the referenced work (Chang *et al.,* 1999) reveals no mention of MDI measurements. The absence of an exposure to MDI is further supported by the observation that no MDA was detected in the hydrolyzed urine of school children purportedly exposed to MDI. The extreme difference in volatility between xylene and MDI, the high xylene content compared to MDI in the applied product (0.1% MDI in xylene), as well as the symptoms consistent with xylene or other solvent exposure, indicate that the symptoms observed were most likely due to the inhalation of xylene.

OEHHA should remove this reference as an example of "toxicity to infants and children" and as a basis for childhood exposure and sensitivity to diisocyanates. The Reactive Airways Dysfunction Syndrome (RADS)-like effects (*e.g.,* dyspnea, cough, headache) seen can be attributed to the irritating and highly volatile solvent, xylene, that was also present.

Response to ACC Comment 5:

Regarding part (a) of ACC's comment, OEHHA also noted in the REL summary that air monitoring was not conducted for MDI (or xylene). However, the authors report that they conducted a simulated spraying operation of the mixture that contained MDI levels of 870 ppm w/w in xylene. Considering that ppb levels can cause respiratory effects, it seems plausible that a spraying/paving operation could result in significant MDI exposure, as well as significant xylene exposure, to children in school classrooms less than 100-240 meters downwind of the operation. To clarify this matter, OEHHA has added more details about the exposure and added information about the simulated spraying results to Section 5.2 of the REL document.

Regarding part (b) of ACC's comment, OEHHA reviewed the Chang et al. (1999) study. This study does not appear relevant to the results of Jan et al. (2008) because Chang et al. were measuring VOC off-gassing from tracks after they were installed, not during application of the tracks. In addition, Chang et al. did not measure any emissions from a track installation operation that consisted of MDI mixed in xylenes. Further, it is unclear if Chang et al. even attempted to measure emissions of isocyanates from track surfaces. OEHHA agrees with ACC that the assertion by Jan et al. that, "Adjacent to such tracks, air levels of MDI were easily detectable even after the first week of track installation" was not discussed in Chang et al. as reported in their study. One possibility for this discrepancy is that Jan et al. may have included the wrong reference in their reference section.

The comment by ACC that "…no MDA was detected in the hydrolyzed urine of school children purportedly exposed to MDI" is true. However, the authors attributed this finding to the short exposure time of the children. Urine sample collection was also delayed until three days following the exposure incident. OEHHA has added the following sentence to address this finding:

"A spot urine test did not reveal a positive reaction for MDA after hydrolyzation of the urine samples. The authors attributed this finding as characteristic of a brief exposure to MDI."

The final comment by ACC is that the extreme difference in volatility between xylene and MDI would support xylene as the major cause of the respiratory symptoms in the children. OEHHA notes that the extreme difference in volatility is somewhat balanced out by the extreme difference in toxicity between the two chemicals. The OEHHA acute REL for xylenes is 22,000 µg/m^3 whereas the proposed acute REL for MDI is 6 µg/m^3 (0.6 ppb). The vapor pressure for xylenes is about 8 mm Hg at 25°C whereas the vapor pressure for MDI is 5×10^{-6} mm Hg @ 25°C. Jan et al. described the track application process briefly as a spraying operation. Thus, the volatility issue raised in the comment may be of little consequence because both xylene and MDI are essentially aerosolized upon release and may have reached the school rooms in roughly equal proportions as found in the original emission source.

Finally, the OEHHA acute REL for xylenes is for nervous system, and eye and respiratory irritation. The reports of dizziness by the children could be due to exposure to xylenes. However, no evidence could be found in the literature that acute exposure to xylenes causes RADS-like effects as ACC suggests. OEHHA will add the following sentences, *"The authors assumed all the symptomology was due to MDI even though xylenes also cause acute eye and respiratory symptoms. Thus, some proportion of the eye and respiratory effects could have been caused by xylene exposure."*

ACC Comment 6:

OEHHA uses a postulation by Krone (2003) and Krone and Klinger (2005) to purport a relationship between polyurethane products and childhood asthma (Section 5.2, 6.2 pgs 10, 24)

OEHHA relies on a study by Krone who reported extracting TDI from foam using a solvent but did not consider an earlier study (Hugo *et al.,* 2000) showing that under more relevant conditions free isocyanate is not emitted from foam (detection limit ~ 0.1 ppb (v/v) in air) even when the foam is purposely loaded with free TDI to ~ 1 ppm (w/w).

Another study compares the use of solvents which actually break the matrix of the foam (Vangronsveld *et al*., 2013). The analysis revealed that the Krone study, that deviated from the prescribed method as proposed by the manufacturer of the tests, other researchers did not confirm Krone results which seems to be caused by breaking down the PU matrix, a scenario impossible in real life as consumers do not use these types of solvents in combination with PU.

Recent references by Arnold *et al.,* 2012, Vangronsveld *et al.,* 2013 were not taken into consideration showing no emission or migration of TDI from foam mattresses. OEHHA

should also review the study by the Danish Authority on MDI in polyurethane products (Boyd & Mogenson, 2007). The findings in these additional references are consistent with work done earlier by California EPA when it detected no TDI from residential foam products even when subjected to elevated temperatures and loading conditions.

OEHHA should review all current literature and evaluate the probability that exposure to MDI and other diisocyanates may occur through inhalation and dermal contact with polyurethane products, early in life or otherwise is extremely low.

Response to ACC Comment 6:

OEHHA has revised the first paragraph of Section 6.2 to include the findings of Hugo et al. (2000), Vangronsveld et al. (2013) and Boyd & Mogenson (2007), taking into consideration that detectable levels of air emissions have not been found from products made with MDI and TDI (this was already noted by OEHHA), and that solvent extraction techniques used to assess release of free diisocyanates may cause decomposition of the test material to form free MDI or TDI. The new paragraphs now read as follows:

"No studies of the chronic effects of MDI on infants and children were located. It has been postulated that early life exposure to TDI and other diisocyanates may occur through inhalation and dermal contact with polyurethane products (Krone et al., 2003). However, emission of detectable levels of free MDI and TDI from polyurethane consumer products and other products made with MDI (e.g., mattresses, adhesives, sealants and other flexible foam products for consumer use) has not been found (Hugo et al., 2000; Boyd and Mogensen, 2007). Strachan and Carey (1995) found independent associations between severe wheeze and the use of non-feather bedding, especially foam pillows (odds ratio 2.78; 95% C.I. 1.89 to 4.17), among children with 12 or more wheezing attacks in the previous 12 months. The authors speculated that volatile organic compounds could be off-gassing from the foam pillows. Other researchers found that there is increased exposure to house dust-mite allergen from synthetic pillows compared to feather pillows and speculated that this may explain the increased asthma symptoms (Crane et al., 1997).

Krone et al. (2003) applied semiquantitative tests (i.e., wipe test and extraction with dimethyl sulfoxide) for isocyanate to polyurethane products manufactured using TDI, including mattresses, mattress pads, sofa padding, carpet pads and pillows, and detected free isocyanate in consumer products. It was suggested by the authors that isocyanate may be available to dissolve in skin oils upon dermal contact. A study by Vangronsveld et al. (2013) used various solvent systems and detection methods to extract free TDI from flexible polyurethane foam. A toluene-based extraction technique was deemed the most consistent and resulted in microgram per gram levels of free TDI extracted from the foam. The authors concluded that the TDI extracted from foam may have been due to decomposition of parts of the foam structure by the solvent, a process that is unlikely to occur under typical household uses. Similar wipe tests and extraction studies on products made with MDI have not been found in the peer-reviewed literature."

The last three paragraphs under Section 6.2 were revised to better address the immune response resulting from TDI/MDI exposure The same material was presented in Section 6.2 of the TDI REL document and has been modified in the same fashion.

ACC Comment 7:

The OEHHA document correctly summarizes the acute animal studies and mentions that the particle size of the exposure atmosphere was < 5 µm (Section 5.3, pgs 10-11).

OEHHA should mention that only about 12% of the particles in an exposure atmosphere of MDI in the workplace would be < 5 µm and considered the thoracic fraction. A respirable fraction of less than or equal to 12% was verified by calculating the percent respirable MDI detected in those cases where sufficient high levels of respirable MDI were detected, i.e., in the spray results and comparing them to the total [vapor + total particulate]. The atmospheres generated for the studies concentrated the small particles by extreme laboratory techniques (Pauluhn, 2008).

PMDI is a liquid with a very low vapor pressure (saturated vapor concentration [SVC] at 20°C is 12 ppb). The Acute Toxicity Inhalation (dust/mist) Category 3 LC50 cut-off of 500 mg/m3 (which represents approximately 50 ppm for PMDI) is over 2500-fold above the SVC for PMDI. Therefore, the intrinsic acute inhalation toxicity of PMDI in the form in which it is most likely to occur (vapor) is very low. PMDI does not occur in particle form (of any particle size) as sold. It is only with processing (i.e., heating, spraying and size screening) that MDI can be modified to a form (i.e., respirable dust/mist) that has measurable acute inhalation toxicity. The toxicity observed appears to require the presence of high concentrations of respirable particles of PMDI. Thus, the question becomes: are the atmospheres generated with MDI for toxicology testing representative of the intrinsic physical/chemical properties of MDI?

A recent study (Vangronsveld and Ahrika, 2014) investigated the concentration of respirable MDI during a wide variety of workplace applications involving PMDI. While the study found detectable levels (detection limit = 0.00002 – 0.0004 mg 4,4'-MDI /m3) of respirable (<4µm) fractions of PMDI aerosols, only six of the nineteen applications monitored produced atmospheres above 0.001 mg/m3 and only two were above 0.010 mg/m3: 0.081 and 0.202 mg/m3 for two spraying operations. To put it more simply, even the highest levels of respirable MDI aerosol (found in workplaces where spraying applications were conducted) are a factor of 2400 (490/0.202) below the 4-hour acute LC50 of Appelman and De Jong (1982). The concentration of respirable MDI for the majority of the remaining applications monitored was more than 240,000 times lower than the 4- hour acute LC50. Although this study may not represent all of the potential spraying applications, the applications monitored are typical of spraying operations involving PMDI. These data are further evidence that the atmospheres generated for the animal studies do not represent the intrinsic physical/chemical properties of PMDI.

Response to ACC Comment 7:

OEHHA does not have access to the Vangronsveld and Ahrika (2014) paper as it is still in preparation according to the ACC reference list. Nevertheless, OEHHA has included recent studies of workplace exposure to MDI, and the Vangronsveld and Ahrika reference may add to what is already discussed in Section 3 (Major Uses and Sources). We already note in the REL document that the vapor pressure of MDI and PMDI are very low, and that, *"Occupational exposure most commonly occurs during processes or applications in which the chemical is sprayed (mainly as an aerosol) or heated."*

OEHHA agrees that the workplace atmospheres of MDI are considerably below the concentrations in LC50 animal studies. However, OEHHA is not primarily focused on the LC50; we are interested in the level of acute, repeated 8-hour, and chronic exposures that result in an level at or below which adverse noncancer health effects are not expected to occur in a human population, including sensitive subgroups (e.g., infants and children). For MDI, the adverse effects on which the RELs are based are respiratory irritation/inflammation and/or lesions to respiratory tissue. Our proposed RELs range from 0.08 to 6 $\mu g/m^3$, which is well within levels generated during workplace operations.

ACC Comment 8:

The statement "[a]t the higher aerosol levels, the lungs of rats euthanized immediately after exposure were grayish and wet, with some pulmonary hemorrhaging and hemorrhagic nasal discharge" appears to indicate nasal discharge from the lungs. (Section 5.3, pgs 10-11)
The Panel suggests modifying the statement to: "At the higher aerosol levels, *hemorrhagic nasal discharge was observed* and the lungs of rats euthanized immediately after exposure were grayish and wet, with some pulmonary hemorrhaging."

Response to ACC Comment 8:

OEHHA has revised the sentence as suggested by the commenter.

ACC Comment 9:

OEHHA references Piirila *et al.* 2000 stating *"[a] 10-year follow-up … found a generally poor medical outcome … of the patients 82 percent still experienced symptoms of asthma, 34 percent used no medication and 35 percent were on*

***regular medication. However, FEV1 reduction did not exceed the predicted decline over time in either smoking or nonsmoking patients*.”**
Piirila (2000) reported symptoms and use of asthma medications at follow-up, however 15% of the surveyed population acknowledged continued work with diisocyanates after being diagnosed and the average duration of symptoms before diagnosis was over 3 years. Prognosis of those with diisocyanate respiratory sensitization is variable. With some, asthma resolves after removal from the isocyanate exposure, but in others it may persist. A favorable prognosis is more likely for those diagnosed with better lung function, milder degree of NSBH, an early reaction (as opposed to a late reaction), and shorter duration of symptoms (Ott *et al*., 2003). Therefore, it is imperative that once diisocyanate related asthma develops, further exposures be fully avoided.

Response to ACC Comment 9:

Much of the information summarized in Piirila et al. (2000) and shown in Comment #9 is already contained in the REL document under Section 6.1. OEHHA will add to the summary that, “…*the average duration of symptoms before diagnosis was over 3 years in these workers.*” OEHHA thanks the commenter for the concise description based on the review by Ott et al. (2003) of outcomes for a favorable prognosis, and will include below the summary of Piirila et al. (2000), “*Prognosis of those with diisocyanate respiratory sensitization is variable. With some, asthma resolves after removal from the isocyanate exposure, but in others it may persist. A favorable prognosis is more likely for those diagnosed with better lung function, milder degree of bronchial hyperreactivity, an early reaction (as opposed to a late reaction), and shorter duration of symptoms (Ott et al., 2003). Therefore, it is imperative that once diisocyanate related asthma develops, further exposures be fully avoided.”*

ACC Comment 10:

OEHHA references Piirila *et al.* 2000 on outcome of diisocyanate asthma but omitted several other studies that provide a more clear picture of the variables that influence outcome of diisocyanate asthma. (Section 6.1, pg 16).
Several authors have correlated prognosis with duration of exposure after symptoms develop. The following table provides correlation between mean of years of symptomatic exposure (YSE) and prognosis of asthma.

Author	**Recovered (YSE)**	**Improved (YSE)**	**Not/Improved (YSE)**
Pisati *et al*., 93	12 (1.6y)	10 (2.8y)	21 (5.4)y
Park 97	17	11	7
Tarlo *et al*., 97	23 (2 y)	60 (2.7y)	18 (4.4)
Pisati *et al.*, 07	10 (.6y)	8 (2.1 y)	7 (4 y)

Tarlo and Liss (2002) state that *"Compared with OA caused by other agents, those with OA due to diisocyanates … severity was milder as assessed by medication use and pulmonary function. Those with diisocyanate-induced asthma were significantly less likely to be hospitalized for asthma. Among the subset whose outcome was determined at a mean of 2.1 years after the main medical assessment, the outcome severity was less for those with diisocyanate-induced OA."*

Outcome at a mean of 1.9 years after initial assessment was significantly better in those with OA induced by isocyanates; 73% cleared or improved versus 56% with other causes of OA ($P < 0.05$) (Tarlo *et al.*, 1997).

In summary, after removal from further exposure, the majority of individuals with diisocyanate related asthma show improvement or totally recover. There is a strong correlation with duration of exposure and at least in one study, it has been suggested that medical surveillance affects recovery. This indicates that lack of recovery is not an unavoidable outcome but can be influenced by early detection through raising awareness, worker education and medical surveillance.

Response to ACC Comment 10:

The intent of the introductory material in Section 6.1 was, in part, to give a brief overview of the level of recovery that can occur following worker sensitization to diisocyanates, and what factors improve or diminish the recovery. Thus, we choose a recent study (Piirila et al. 2000) with a long-term follow-up to represent this topic. The current summary and the additional information regarding outcome of diisocyanate-induced asthma that we included (see Response to ACC Comment 9) at the suggestion of the commenter should be sufficient to give the reader a good understanding of the potential for recovery following sensitization to diisocyanates.

ACC Comment 11:

OEHHA reviewed Petsonk *et al.* 2000 and correctly states that the study was based on questionnaires and *"[t]hus, the authors noted that it was unlikely that all participants with respiratory symptoms have occupational asthma."* (Section 6.1, pg 17).

Although OEHHA correctly summarized the study, the inclusion of statement on page 22 is misleading, *"[o]f 178 workers, 12% had new onset of asthma after 2 years related to those working in high exposure areas ($p<0.001$). The paper's objective was to evaluate the questionnaire as a tool for medical screening and it concludes that it is a valid epidiomological method with variable sensitivity and specificity … Screening examinations must be followed by a rigorous and systematic evaluation."* The Panel does not consider this study as evidence paper for MDI toxicity since it is evaluating a questionnaire and not the relevant procedure for diagnosing occupational asthma.

Response to ACC Comment 11:

OEHHA thanks the commenter in pointing out the error in Table 2 on page 22. The statement now reads, *"15 of 56 workers with high exposure had new onset of asthma after 2 years vs. 0 of 42 workers with low exposure (p<0.001)."*

There are relatively few epidemiological studies in the literature that evaluated the effects of MDI exclusively as Petsonk et al. (2004) does, so we believe the study should be included in the REL summary. Other advantages are that this was a prospective study carried out concurrent when MDI exposures began, and spirometric and immunologic testing was performed during the survey that tended to confirm the validity of the asthma-like symptoms reported on the questionnaires. The study protocol was also reviewed and approved by the National Institute for Occupational Safety and Health. Regarding the questionnaire itself, the Materials and Methods sections notes that*, "The survey included administration of a respiratory health questionnaire comprised of elements from previous standard instruments (British Medical Research Council, 1976)."*

The commenter seems to suggest this study should have performed provocation tests on the workers with asthma-like symptoms to be relevant. The gold standard of positively confirming a clinical diagnosis of diisocyanate-induced asthma through specific inhalational challenge in exposure chambers is expensive, and must be calibrated and validated. This is not always available to all researchers. There is also the very real prospect of a false negative even with specific inhalational challenge. In practice, diagnosis usually depends on documentation of bronchial hyperreactivity and a positive association of symptoms and physiologic changes with exposure (Liu and Wisnewski, 2003), as Petsonk et al. (2000) attempted to do. Despite the limitations of the Petsonk et al. study that we have already outlined in the summary, OEHHA believes this study is important to present in the MDI REL document.

Reference: Liu Q and Wisnewski AV. 2003. Recent developments in diisocyanate asthma. Ann Allergy Asthma Immuno 90(suppl):35-41.

ACC Comment 12:

OEHHA uses a reference by Reidy and Bolter (1994) to suggest neurological effects. (Section 6.1, pg 23).

OEHHA failed to review the recent publication on neurotoxicity (Hughes *et al*. 2014) which reviews the Reidy and Bolter study as follows: "Major limitations of this report include strong selection bias, lack of comparison with other exposed workers, and a lack of quantitative data on exposure to MDI and other concomitant agents. Potential confounders also limit conclusions, as the authors concede findings could be due to emotional stress and potential impact of compensation bias in the test results.

Regardless, testing was largely normal except for the presence of mood disorder in all subjects and mild abnormalities in memory learning. Thus, given these extensive limitations and the lack of specific findings, the data presented does not provide evidence of MDI neurotoxicity."

The paper concludes that *"[t]here is insufficient evidence for a causal association of neurotoxic effects and diisocyanate exposure based on lack of evidence in all categories of the Hill criteria for causality except for temporal association of reported symptoms and alleged exposure. Future reports should attempt to address more rigorous exposure assessment and control for confounding exposures."*

OEHHA should correct or remove the statement *"There are also case reports of neurological effects"* as a systematic review of the literature evaluating the causal association on humans does not support this alleged association.

Response to ACC Comment 12:

We note in the MDI REL document that there are limitations to this study that are presented by the authors, including concomitant exposure to other solvents, the lack of intensity and frequency of exposures, and that a single pattern of neuropsychological deficits associated with MDI exposure could not be found. We will include the following paragraph with additional weaknesses of the study highlighted in Hughes et al. (2014):

"Hughes et al. (2014) reviewed the study by Reidy and Bolter (1994), along with a number of other studies suggesting neurological deficits resulting from exposure to other diisocyanates. They purport that the Reidy and Bolter study was biased as a result of testing obtained by litigating attorneys, and that there was a lack of comparison with other exposed workers. They also point out that the authors say selection bias was present, as there were other workers exposed to MDI who refused to participate for various reasons."

We have also modified the first sentence in our summary of the study, as suggested by the commenter.

ACC Comment 13:

OEHHA incorrectly states that *"[t]he presence of MDA in urine was explained, in part, by the long half-life of MDA in the body, and that exposure from previous days contributed to the urinary amount of metabolite."* (Section 6.1, pg 23)

OEHHA correctly states *"MDA is formed following acid hydrolysis of MDI metabolites in urine samples and is preferred for quantitative analysis"* (in Section 4, pg 5) yet continues to call MDA a metabolite of MDI (discussed in Section 1.a. of these Comments) and discusses the presence of MDA in the body.

"The current state of knowledge of MDI metabolism: the absence of any detectable MDA in a mass balance study where a dose of MDI substantially higher than that permitted for workers was used; the major route of metabolism is via N-acetylation; the identified metabolites can be derived from MDI without invoking the presence of MDA; the biochemical studies lead to an understanding that hydrolysis is the least preferred mechanism under physiologic conditions; and the clarification of biomonitoring data where weak-base hydrolysis likely led to MDA formation ex vivo. Therefore, the weight of evidence leads to the conclusion that if MDA is formed at all following inhalation exposure of humans, it occurs at currently undetectable amounts and should be considered negligible from a toxicologic perspective."

Response to ACC Comment 13:

The paragraph in question was modified to clarify that MDI metabolites are present in the urinary samples, and that acid-hydrolyzed urinary samples result in measurement of these MDI metabolites as MDA.

ACC Comment 14:

OEHHA incorrectly uses a study by Krone *et al.* (2003) to suggest that *"early life exposure to MDI and other diisocyanates may occur through inhalation and dermal contact with polyurethane products."* (Section 6.2 pgs 23-24)
See Comment 2(b) above for a more detailed explanation.

Response to ACC Comment 14:

This sentence as well as the entire paragraph was revised. Please see "Response to ACC Comment #6" above. The sentence in question now says, "*It has been postulated that early life exposure to MDI and other diisocyanates may occur through inhalation and dermal contact with polyurethane products (Krone et al., 2003)."*

ACC Comment 15:

For the acute, 8-hr and chronic RELs (Sections 8.1, 8.2 and 8.3, pgs 38-44), the use of a √10-fold interspecies toxicodynamic (TD) UF for metabolic variability is inappropriate.

The observed effect on the pulmonary epithelium is considered to be the result of a direct acting irritant rather than an indirect effect dependent on metabolism to produce an adverse outcome. This conclusion is based on reports that direct acting irritants

administered to rodents typically induced lesions in the olfactory epithelium and in the respiratory epithelium (Jiang *et.al*., 1983; Gaskell, 1990; Abdo *et al*., 1998) whereas indirect acting chemical compounds typically induce changes in the olfactory epithelium while sparing the respiratory epithelium (Gaskell, 1990). For MDI, the findings of histopathologic changes in the respiratory and olfactory epithelium are consistent with a direct acting irritant therefore the √10-fold interspecies toxicodynamic (TD) UF for metabolic variability is inappropriate.

Response to ACC Comment 15:

A default interspecies toxicodynamic (TD) UF of √10 is applied when there is no data on TD interspecies differences, whether or not the chemical is a direct or indirectly acting agent on respiratory epithelial tissue. This is consistent with our default uncertainty factor approach used in deriving RELs (OEHHA, 2008). The application by OEHHA of a TD UF = √10 can also be found in the derivation of other RELs in which the critical endpoint is olfactory or respiratory epithelial lesions, including acetaldehyde, acrolein, caprolactam, and others. It should be noted that using benchmark dose methodology, we found that the critical endpoint in rodents was pulmonary epithelial lesions, with olfactory lesions in the upper respiratory airways being slightly less sensitive. We therefore based the RELs primarily on the lower airway lesions rather than the upper airway lesions.

ACC Comment 16:

For the acute, 8-hr and chronic RELs (Sections 8.1, 8.2 and 8.3, pgs 38-44), uses an intraspecies toxicodynamic (TD) UF of 10 based on the following rationale: (a) genotypic variation in MDI metabolizing enzymes, (b) MDI's sensitizing potential, and (c) greater susceptibility of children to the asthma exacerbating effects of MDI as described by Jan *et al*. (2008).

Genotypic variations in metabolic enzymes are not relevant to MDI (see above). OEHHA provides no evidence that RELs developed on the basis of the critical (most sensitive) effects are not protective of neuroimmune sensitization. The Jan *et al*. (2008) article does not support the contention that MDI exacerbates asthmatic symptoms in children (see above). Based on data in animals and humans, the Th1 / Th2 hypothesis discussed by OEHHA in its TDI REL document predicts that asthmatic children should be less sensitive – not more sensitive - to the sensitizing effects of diisocyanates.

Response to ACC Comment 16:

Genotypic variation in metabolic enzymes, and antioxidant defense, was part of the reason for using an intraspecies toxicokinetic UF = 10. Genotypic variation in enzymes and factors involved in immune regulation and inflammatory regulation were also investigated. These are more pertinent to intraspecies toxicodynamic properties and

used as part of the reasoning for an intraspecies TD = 10. This is explained in Response to ACC Comments #3 and #4 above, and # 19 below. Briefly, it is suspected that genotypic variation of GST metabolizing enzymes and other enzymes are important to the disease process caused by MDI exposure. A number of gene variants have been reported to be associated with increased sensitivity to the disease in workers, which suggests that diisocyanate-induced asthma represents a complex disease phenotype determined by multiple genes.

As outlined in Response to ACC Comment #5, we believe MDI could indeed have caused the RADs-like symptoms in the Taiwanese children acutely exposed to MDI. We agree with ACC that some proportion of the toxic response could have resulted from exposure to the solvent xylenes. We have added additional information suggesting acute xylene exposure could be involved in the response. Irrespective of the Jan et al. (2008) study, OEHHA increases the default intraspecies toxicodynamic UF from √10 to 10 for chemicals that are sensitizers and for the known greater susceptibility of children to the asthma-exacerbating effects of such chemicals. For example, we used an intraspecies toxicodynamic UF = 10 for the formaldehyde RELs to address potential asthma exacerbation in children.

The comment that children should be less sensitive – not more sensitive – to the sensitizing effects of diisocyanates because childhood asthma is Th2-driven (as opposed to diisocyanate sensitization which can be Th1-driven) is not adequately supported by the available data. It is unknown how children will react to MDI and TDI exposure early in life when the immune system is still developing. The development of asthma from exposure to MDI and TDI is multifactorial and it is not well understood what the detailed mechanism for diisocyanate-induced asthma is in adults, much less children. A revised discussion of the immune response in childhood atopic asthma and diisocyanate asthma is presented in Section 6.2. Uncertainty factors are assigned based on data gaps, and the lack of knowledge regarding the relative susceptibility of infants and children compared to adults represents a substantial data gap. Thus, we assigned an intraspecies toxicodynamic UF = 10, in part, for what is unknown about chemically-induced asthma in children. Further, OEHHA considers asthma to be a disease that disproportionately impacts children. Thus, whether MDI induces or exacerbates asthma in children, we would use a higher toxicodynamic uncertainty factor to protect children, as we have for other RELs.

ACC Comment 17:

The 8-hr REL was derived by OEHHA (Section 8.2, pg 42) using a time- adjusted exposure concentration calculated in a manner inconsistent with OEHHA guidance and practice.

OEHHA's time adjustment factor (6 hrs/24 hrs x 5 days/7 days x 20 hrs/10 hrs) is inconsistent with its 2008 REL guidance document (Chapter 6). The time- adjustment factor for an 8 hr REL should be the rat exposure duration (6 hr/ 8 hr) multiplied by the

rat exposure frequency of 5 days / 7 days to convert the less than daily exposures to a continuous (daily) exposure. The incorporation of 20 hrs / 10 hrs (sic), which should actually be 20 m3/day/ 10 m3/day, is inappropriate and should be deleted. Although the ratio of 20 / 10 could be used to convert a chronic (24 hr) human exposure to an 8 hr workplace exposure, this is not the exposure in question. It would appear that OEHHA is mixing rodent and human exposure approaches in a less than transparent manner to reduce the standard time- adjustment factor of 0.54 (6 hrs/24 hrs x 5 days/7 days) to 0.36 (6 hrs/24 hrs x 5 days/7 days x 20 hrs/10 hrs).

Response to ACC Comment 17:

OEHHA thanks the reviewer for pointing out the 20 hr/10 hr factor shown in the draft MDI document should actually be shown as 20 m^3/10 m^3. OEHHA has used the 8-hour time adjustment, which includes the 20 m^3/10 m^3 factor, based on intermittent exposures in an animal study to derive 8-hour RELs for worker exposures.

Our Hot Spots Noncancer Guidelines (OEHHA, 2008) show that in cases where an 8-hour REL should be derived based on chronic exposure, it is appropriate to use the 20m^3/10m^3 conversion:

"*Based on the assumption that half of the 20 m^3 of air breathed in any 24-hour period is breathed while active at work, the default approach to estimating an equivalent inhalation-weighted average concentration (C_{AVG}) for an eight-hour period of elevated activity (such as at work) from the observed concentration (C_{OBS}) for continuously exposed humans or experimental animals is:*

$C_{AVG} = C_{OBS}$ *x (20 m^3/day total exposure / 10 m^3/day occupational exposure) x (D days per week)*

Commonly encountered exposure scenarios in both worker studies and experimental animal toxicology studies involve exposures of 6 to 8 hours per day for 5 days per week. Less time adjustment, and associated uncertainty, occurs applying an eight-hour REL under these exposure scenarios relative to applying a chronic REL."

Thus, we also use the worker daily inhalation conversion factor to derive 8-hour RELs from animal studies where the animals were exposed intermittently (e.g., 6 hrs/day) on a daily or near daily basis (e.g., 5 days/week).

For example, both our acrolein and acetaldehyde 8-hour RELs are based on rat studies in which the animals were exposed 6 hours/day, 5 days/week for 4-6 weeks. We extrapolated to an 8-hour concentration using the conversion:

$$6 \text{ hr}/24 \text{ hr} \times 5 \text{ days}/7 \text{ days} \times 20 \text{ m}^3/10 \text{ m}^3$$

This is the same conversion used in the 8-hour REL derivation for MDI. We state in our acetaldehyde REL derivation (OEHHA, 2008, Appendix D1) that:

"The time adjustment for an 8-hour REL used is 6h/24h × 20 m^3/10 m^3, rather than 6 h/8 h, because we assume that the 8 hours includes the active waking period when an adult inhales 10 m^3 of air, i.e. half the daily total intake of 20 m^3."

ACC Comment 18:

For the 8-hr and chronic RELs (Sections 8.2 and 8.3, pgs 41-44), OEHHA should transparently indicate that its selection of a 5% benchmark response (BMR) is a policy decision that results in a 3-fold lower BMCL than was calculated by USEPA which used a 10% BMR to derive a REL-like value (RfC) for MDI from the same dataset.
If the 5% criterion is retained, a less conservative policy should be used when considering other UFs to produce a more balanced assessment.

Response to ACC Comment 18:

OEHHA presents our use of the 5% benchmark response (BMR) in our Noncancer Guidelines (OEHHA, 2008) and cites supporting documentation showing why the 5% BMR appears to be equivalent to a NOAEL in well designed and conducted animal studies. Therefore, we believe a less conservative use of UFs is not appropriate when using the 5% BMR for deriving 8-hour and chronic RELs. Specifically, we state in our Noncancer Guidelines (OEHHA, 2008):

"A response range of 1% to 5% approximates the lower limit of adverse effect detection likely to occur in typical human epidemiological studies, and in large laboratory animal studies the detectable response rate is typically in the 5 to 10% range (Gaylor, 1992). In 1995, using animal developmental toxicity data, the U.S. EPA concluded that a 1% response rate was likely to be too low to be detected and therefore too uncertain to use as a point of departure, while either 5% (BMC_{05}) or 10% (BMC_{10}) response rates were adequate for the purposes of estimating a benchmark concentration (Barnes et al., 1995). One reason for this conclusion was the large difference (29-fold) between observed NOAELs and the 1% benchmark using developmental toxicity data. Subsequently, the U.S. EPA (2007a) used a 10% response rate for benchmark concentrations when deriving chronic inhalation reference concentrations (RfCs). More recently, RfC determinations for various endpoints by the U.S. EPA have used either 5% or 10% as the benchmark response rate, depending on the statistical uncertainty in the data (U.S. EPA, 2002a; U.S. EPA, 2004). OEHHA has used the 5% response rate in several chronic RELs, and showed that the lower 95% confidence bound on the BMC_{05} typically appears equivalent for risk assessment purposes to a NOAEL in well designed and conducted animal studies where a quantal measure of toxic response is reported (Lewis and Alexeeff, 1989; Alexeeff et al., 1992; Alexeeff et al., 1993; Barnes et al., 1995; Collins et al., 2004; Collins et al., 2005; Starr et al., 2005; Alexeeff et al., 2006; Brown et al., 2006). Therefore, OEHHA typically uses a 5% response rate as the default

for determination of the BMC from quantal data (i.e. the effect is either present or it is not) in animals (Fowles et al., 1999)."

ACC Comment 19:

For the 8-hr and chronic RELs (Sections 8.2 and 8.3, pgs 41-44), the use of a 10-fold intraspecies toxicokinetic (TK) UF for metabolic variability is inappropriate and inconsistent with available data and past REL practices.

The role for genotypic variation in glutathione transferases (GSTs) is negated by the fact that GSTs are not required for the reaction of MDI with glutathione (Day *et al*., 1997). The effects noted in rats are likely due to the ability of MDI to bind to cell membrane proteins in the pulmonary epithelium. Toxicokinetics, and genotypic variations in metabolic enzymes in particular, do not play a role in these direct effects on the olfactory epithelium. Thus, a TK UF greater than 1 is not justifiable.

Response to ACC Comment 19:

This comment is related to Comment #3 above, in that the commenter suggested metabolic enzymes including GSTs are not important to the disease process caused by MDI exposure. Diisocyanates or their metabolites may react with intracellular glutathione (GSH), either directly or after catalysis by the GSTs. Thus, GSTs may help facilitate the reaction of GSH with MDI. Exposure to diisocyanates including MDI causes respiratory symptoms characterized by airway inflammation, eosinophilia, and local formation of reactive oxygen species (ROS). Piirila et al. (2001) notes that enzymes of the GST supergene family can utilize a wide variety of products of oxidative stress as substrates and are thus critical in the protection of cells from ROS. Accordingly, the observed wide genetically based individual variations in the GST enzyme activities are candidates as modifiers of susceptibility to diisocyanate-induced asthma. Individual capability to tolerate oxidative stress varies, possibly due to genetic factors. Inability to detoxify ROS could therefore lead to inflammatory process, activate bronchoconstrictor mechanisms and cause asthmatic symptoms.

In addition to GSTs, a number of other gene variants have been reported to be associated with increased sensitivity to the disease in workers, which suggests that diisocyanate-induced asthma represents a complex disease phenotype determined by multiple genes. Examples of genes shown in Table 9 of our MDI REL document include, but are not limited to, genes involved in immune regulation (human leukocyte antigen, cytokines IL4RA, IL-13, and CD14), inflammatory regulation (alpha-T catenin), and other genes involved in antioxidant defense (superoxide dismutase, epoxide hydrolase). The mean Odds Ratios for significant genotype variation associations and increased susceptibility for diisocyanate-induced asthma were between 1.89 and 10.36, based on metabolic enzymes including GST, NAT, and EPXH. This would suggest there could be a large (up to 10-fold) variation in the human pharmacokinetic response.

Thus, a 10-fold intraspecies toxicokinetic uncertainty factor is appropriate for risk assessment. Further variation in other genes associated with the inflammatory process and immune regulation also demonstrated associations with diisocyanate-induced asthma (OR between 2 and 9). Thus, this supports use of a toxicodynamic factor greater than the default of √10.